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Cansjera rheedii J. F. Gmel. a medicinal plant-mediated synthesis of silver nanoparticles and their antibacterial activity

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Abstract:

The laboratory experiments were conducted to synthesis silver nanoparticles using the leaf extract of Cansjera rheedii J. F. Gmel.The characteristic Plasmon resonance at 430 nm in UV-Vis spectrophotometer confirmed the formation Silver nanoparticles. The FTIR data reveals the possible functional groups of biomolecules involved in bioreduction and capping for efficient stabilization of silver nanoparticles. HRSEM studies revealed that the nanoparticles are spherical in shape and size ranges between 30 and 50 nm. These silver nanoparticles were evaluated for their antibacterial efficacy against the bacteria Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli. The Silver nanoparticles inhibited the growth of Pseudomonas aeruginosa and staphylococcus aureus but no inhibition was observed in case of Escherichia coli.

Keywords:

Antibacterial, *Cansjera rheedii* J. F. Gmel., Silver Nanoparticles, & HRSEM

Introduction

The synthesis of nanoparticles with the desired quality and properties is one of the key issues in current nanotechnology [1]. The "green" synthesis of metallic nanoparticles has received increasing attention due to the development of eco-friendly technology in material science. Well-dispersed and ultrafine metal nanoparticles especially, transition metals have great interest due to their distinctive physicochemical and thermodynamic properties which have made them suitable for use in various fields such as catalysis, photonics, biomedicine, antimicrobials and optics [2,3,4,5,6,7]. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. Biosynthesis of nanoparticles is advantageous over physical and chemical methods as it is a cost-effective and environment friendly method, where it is not necessary to use high pressure, energy, temperature and toxic chemicals[8,9].The several workers reported the biological synthesis of nanoparticles using bacteria, fungi and plant extracts. Silver nanoparticles have been synthesized using various plant extracts such as *Cinnamon camphora* [10], Geranium [11], Neem leaf broth [12], *Aloe vera* [13], Tamarind leaf [1], *Phyllostachys* sp leaves [14], *Acalypha indica* [15], and *Capsicum annum* [16]. In the present investigation an attempt has been made for biosynthesis of silver nanoparticles using leaf extract of *Cansjera rheedii* J. F. Gmel. and their antibacterial activity tested against gram positive and gram negative bacteria to evaluate their antibacterial efficacy.

Material and methods

Preparation of leaf extract:

Leaves of *Cansjera rheedii* J. F. Gmel. were collected from the Jogimatti State Reserve Forest Chitraduga, Karnataka, India. Leaves were washed 2-3 times with tap water followed by double distilled water to remove dust and impurities. Leaves were shade dried for 5 days and blended using kitchen blender to obtain the powder. The leaf powder was sterilized at 121 ºC for 15 min. 10 g of powder was taken and mixed with 100 ml of double distilled water and kept in shaker for 24 hours. The extracts were filtered through Wattman No1 filter paper and stored in refrigerator at 4 ºC for further use.

Synthesis of Silver nanoparticles:

Five millilitre of the filtrate was added to 250ml Erlenmeyer flask containing 100 ml of 3mM aqueous silver nitrate solution. The mixture was subjected for shaking at rotation speed of 200 rpm for 48 hours at 30°C and the pH was maintained between 6-7. Synthesis of the silver nanoparticles was confirmed by the colour change of mixture from Colorless to dark brown.

Characterization:

The bioreduction of silver ions in the solution at different time intervals was monitored by using Uv-Visible spectrophotometer (U-3010). The solution containing bioreduced silver ions was centrifuged at 6000 rpm for 20 min to remove the unwanted biomass residue, the resulting suspension was then dispersed in 10 ml of double distilled water and centrifuged again at the same conditions. Redispersion and centrifugation process was repeated for 2-3 times to obtain the pellet of silver nanoparticles free from any biomass residue. The pellet thus obtained was dispersed in double distilled water and oven dried to obtain the powder. The powder was used for FTIR, HRSEM and EDAX studies. Scanning electron microscopic studies were carried out at SAIF IIT Chennai using High Resolution Scanning Electron microscope attached with Energy Dispersive X-ray analyzer (EDAX) to confirm the shape, size and elemental nature of nanoparticles.

Antibacterial activity:

The antibacterial assay was carried out on like *Pseudomonas aeruginosa*, *Escherichia coli* (Gram negative) and *Staphylococcus aureus* (Gram positive) by standard disc diffusion method [17]. This method depends on the radial diffusion of an antibiotic, from the disc through semisolid agar layer in Petri plate, which prevents the growth of micro organisms in a circular area or the zone around the disc.

The hot sterile medium was poured into the sterile Petri plates to form 2-3 mm thick uniform layer and allowed to solidify. These plates were later lawn cultured with bacterial broth suspension. The sterile discs impregnated with 5µl, 10µl and 15µl of the extract were placed on the media with sterile forceps and gently pressed down to ensure contact with the suspension. To increase the efficiency (sensitivity) of the test system the plates were kept for 30 minutes at room temperature. The plates were incubated at 37^0 C for 24 hours and zone of inhibition (including the diameter of sterile disc) was measured using Hi-Antibiotic Zone scale.

Results and Discussion:

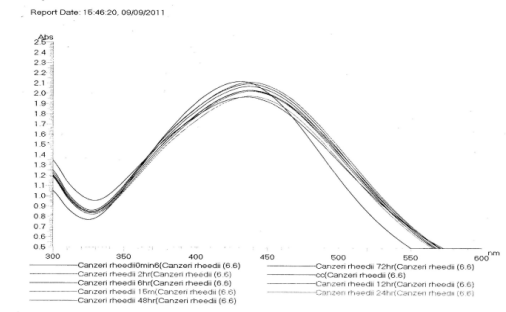
The color change of 3mM aqueous $AgNO_3$ solution from colourless to brown was noticed after 30 minutes of reaction with leaf extract of *Cansjera rheedii* .The colour change is due to excitation of surface plasmon vibrations of silver nanoparticles [18].The intensity of brown colour of the reaction mixture increases with the increase in the time duration up to 72 hours indicating the degree of bioreduction of silver ions in the solution.The surface plasmon resonance of silver nanoparticles produced peak at 430 nm at the interval of 30 minutes of reaction period.

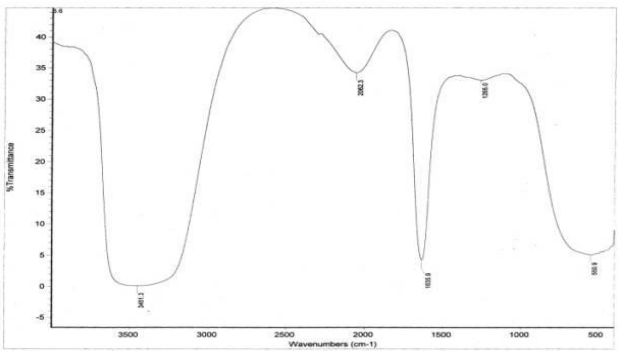
FTIR measurements were carried out to identify the biomolecules responsible for capping and efficient stabilization of the silver nanoparticles. The FTIR spectrum (Fig.2)showed the peaks at 3451.3cm^{-1} , 2062.3cm^{-1}, 1835.9cm^{-1} and 1265.9cm^{-1}.The peaks at 3451.3cm^{-1} and 2062.3$cm^{-1}$are found to be associated with the functional groups of dimeric OH stretch and secondary OH in-plane bend which are assigned to alcohols and hydroxyl compounds. The peak at 1835.9cm^{-1} is associated with five membered ring anhydride (acyl anhydride) functional group of carbonyl compounds. These carbonyl group of amino acid residue have strong binding ability with suggesting the formation of layer covering and acting as capping agent to prevent agglomeration and provide stability [19].The peak at 2062.3cm^{-1} is associated with Isothiocyanate group of nitrogen multiple and cumulated double bond compounds [20].

The HRSEM micrograph of Silver nanoparticles (Fig.3) revealed the spherical shape of silver nanoparticles which ranges between 30 and 50 nm in size. The EDAX spectrum confirmed the purity and elemental nature (Fig.4). Similar result was reported by Singh *et.al.*2011, A green biogenic approach for synthesis of gold and silver nanoparticles using Z*ingiber officinale* [21] and Prathap *et.al.*2006 using *Aloe vera* plant extract' [13].

The antibacterial activity of silver nanoparticles performed against bacteria *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* showed inhibition zones of 9.5mm, 12mm and 21mm for concentrations of 5µl, 10µl and 15 µl respectively against *Pseudomonas aeruginosa*, 8.5mm, 9.5mm and 10mm for concentrations of 5µl, 10µl and 15 µl respectively against *Staphylococcus aureus* but, no inhibition zone was observed for *E.coli* (Fig.5 & 6).Silver ions released by the nanoparticles may attach to negatively charged bacterial cell wall and rupture it, thereby leading to protein denaturation and cell death [22]. Ag+ ions uncouple the respiratory chain from oxidative phosphorylation or collapse the proton-motive force across depending on the size and shape [23]. Morones *et al.* [24] reported that AgNPs preferentially bound to the cytoplasmic membrane leading to cell damage. In addition to that, the pitting caused by AgNPs in the bacterial cell wall is also responsible for the death of bacteria. However, the actual antibacterial mechanism of AgNPs is not well known. Some researchers believe that silver releases Ag+ ions and these interact with thiol groups of bacterial proteins affecting the replication of DNA [25].

Fig1. UV-Vis Absorption spectrum of silver nanoparticles synthesized using *Cansjera rheedii* leaf extract.





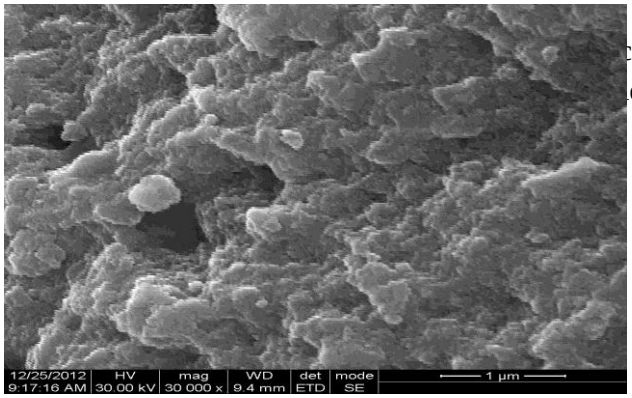


Fig3. HR-SEM micrograph of silver nanoparticles synthesized using *Cansjera rheedii* leaf extract.

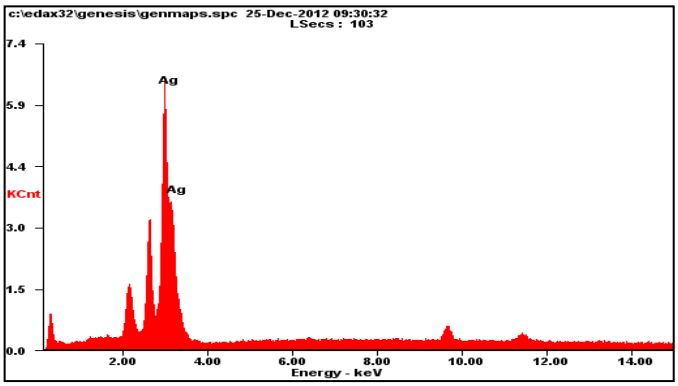


Fig4. EDAX of silver nanoparticles synthesized using *Cansjera rheedii* leaf extract.

Fig.5. Antimicrobial activity of silver nanoparticles synthesized using *Cansjera rheedii* leaf extract.

A.Pseudomonas aeruginosa B.Staphylococcus aureus C.Escherichia coli

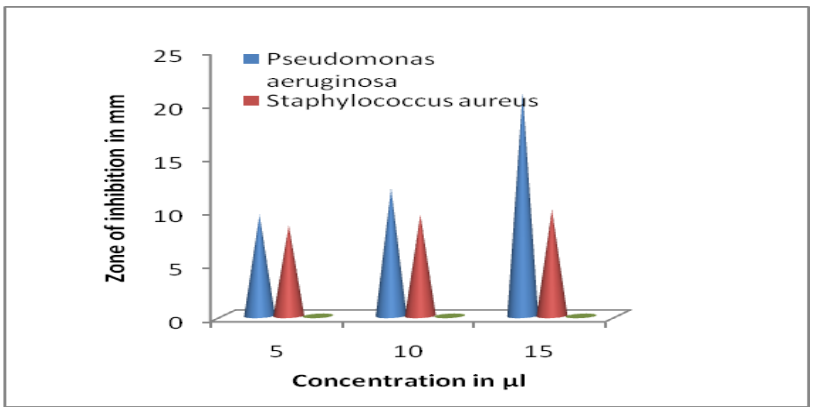


Fig.6.Graph showing the zones of inhibition against the bacteria.

Conclusions:

Cansjera rheedii leaf extract was used for the synthesis of silver nanoparticles. The characterization revealed silver nanoparticles are spherical in shape and range between 30 and 50 nm in size. Elemental analysis by EDAX confirms the purity and elemental nature of silver nanoparticles. They showed good antibacterial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

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References:

i. *B. Ankamwar, M. Chaudhary, and M. Sastry, "Gold nanotriangles biologically synthesized using tamarind leaf extract and potential application in vapor sensing," Synthesis and Reactivity in Inorganic, Metal-Organic, and Nano-Metal Chemistry, Vol. 35, no. 1, pp. 19–26, 2005.*

ii. *C. Wang, N.T. Flynn, and R. Langer, R, "Controlled structure and properties of thermoresponsive nanoparticle-hydrogel composites", Journal of Advanced Materials, Vol. 16, pp.1074 – 1079, 2004.*

iii. *A.Biswas, O.C. Aktas, U.Schumann, U. Saeed, V. Zaporjtchenko, and F. Faupel, "Tunable multiple plasmon resonance wavelengths response from multicomponent polymer-metal nanocoposite systems", Applied Physical Letter, Vol.84, pp.2655 – 2657, 2004.*

iv. *A.N. Shipway and I. Willner, "Nanoparticles as structural and functional units in surface confined architectures",Chemical Communication, Vol.20, pp.2035 - 2045,2001.*

v. *S. Nie and S.R.Emory, "Probing single molecules and single nanoparticles by surface enhanced Raman Scattering",Science., Vol. 275, pp.1102 – 1106, 1997.*

vi. *K. Govindraju, V. Kiruthiga, and G. Singaravelu, "Evaluation of biosynthesized silver nanoparticles against fungal pathogens of mulberry Morus indica", Journal of Biopesticides, Vol.1 No.1 , pp. 101 – 104,2008.*

vii. *K. Govindraju, V. Kiruthiga, V. Ganesh Kumar, and G. Singaravelu, "Extracellular synthesis of silver nanoparticles by a marine alga, Sargassum wightii Grevilli and their antibacterial effects", Journal of Nanoscience and Nanotechnology, Vol.9, pp. 5497 – 5501,2009.*

viii. *Z.P. Xu, Q.H. Zeng, G.Q. Lu and A.B. Yu, "Inorganic nanoparticles as carriers for efficient cellular delivery", Chemical Engineering Science, Vol. 61, pp.1027-1040,2006.*

ix. *S. Goodsell. Bionanotechnology: Lessons from Nature. John Wiley & Sons Inc. Publication 2004.*

x. *J. Huang, Q. Li, D. Sun, Y. Lu, Y. Su, X. Yang, H. Wang, Y. Wang, W. Shao, N. He ,J. Hong and C. Chen, "Biosynthesis of silver and gold nanoparticles by novel sundried Cinnanonum camphora leaf", Nanotechnology, Vol. 18, pp.1-11, 2007.*

xi. *S.S.Shankar, A. Ahmad and M. Sastry, "Geranium leaf assisted biosynthesis of silvernanoparticles", Biotechnol. Prog., Vol.19, pp.1627-1631, 2003.*

xii. *S.S.Shankar, A. Rai, A. Ahmad and M. Sastry, "Rapid synthesis of Au, Ag and bimetallic Au core-Ag shell nanoparticles using Neem (Azadirachta indica) leaf broth.", J. Colloid. Interface. Sci, Vol. 275, pp. 496- 502, 2004.*

xiii. *S. C. Prathap, M. Chaudhary, R. Pasricha, A. Ahmad and M. Sastry, "Synthesis of gold nanotriangles and silver nanoparticles using Aloe vera plant extract", Biotechnol. `Prog, Vol.22, pp.577-583,2006.*

xiv. *A.Jegan, A. Ramasubbu, S. Vasanth kumar, M. Balamurugan and S.Saravanan, "Environmental benign synthesis and characterization of silver Nanoparticles using Phyllostachys sp leaves", Digest Journal of Nanometerials and Biostructures, Vol. 6, No 1,p p. 325-330,2011*

xv. *E.G.Krishnaraj,S.Jagan, P. Rajasekar, Selvakumar, P.T. Kalaichelvan and N. Mohan., "Synthesis of silver nanoparticles using Acalypha indica leaf extracts and its antibacterial activity against water borne pathogens",Colloids and Surfaces B: Biointerfaces, Vol. 76, pp.50, 2010.*

xvi. *S. Li, Y. Shen, A. Xie, X. Yu, L. Qui, L. Zhang and Q. Zhang, "Green synthesis of silver nanoparticles using Capsicum annum L. extrac"t. Green Chemistry, Vol 9, pp. 852 858, 2007.*

xvii. *A.M. Bauer, W. M. Kirby, J. C. Sherries and M. Truck, American Journal of Clinical Pathology, 33(1966) pp.493-496.*

xviii. *P. Mulvaney (1996) Surface Plasmon spectroscopy of nano-sized metal particles. Langmuir 12:788-800.*

xix. *R. Sathyavathi , M. B. Krishna ,S.V. Rao, R. Saritha and D. N. Rao (2010). Biosynthesis of silver nanoparticles using Coriandrum sativum leaf extract and their application in nonlinear optics. Adv Sci Lett 3:1-6.*

xx. *J. Coates. Interpretation of Infrared spectra, a practical approach. Encyclopedia of Analytical Chemistry. R.A. Meyer.(Ed.)2000,pp.10815-10837.*

xxi. *C. Singh, V. Sharma, P. Naik, V. Khandelwal and H. Singh, "A green biogenic approach for synthesis of gold and silver nanoparticles using Zingiber officinale", Digest Journal of Nanomaterials and Biostructures,Vol. 6, No 2, pp.535-542, 2011.*

xxii. *A.R.Nalwade and A.Jadhav, "Biosynthesis of silver nanoparticles using leaf extract of Daturaalba Nees. And evaluation of their antibacterial activity",Archives of applied science research, vol.5 No.3,pp.45-49.*

xxiii. *K. J. Kim, W. S. Sung, S. K. Moon, J. S. Choi, J. G. Kim, and D. G. Lee, "Antifungal effect of silver nanoparticles on dermatophytes," Journal of Microbiology and Biotechnology, Vol. 18, No. 8, pp. 1482–1484, 2008.*

xxiv. *J. R. Morones, J. L. Elechiguerra and A. Camacho. "The bactericidal effect of silver anoparticles", Nanotechnology, Vol. 16, No. 10, pp. 2346– 2353, 2005.*

xxv. *M. Marini, S. De Niederhausern and R. Iseppi , "Antibacterial activity of plastics coated with silver-doped organic-inorganic hybrid coatings prepared by sol-gel processes," Biomacromolecules, Vol. 8, No. 4, pp. 1246–1254, 2007.*